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Influence of Plant Growth Regulators on Management of Barley Powdery Mildew under Salinity Stress

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The effect of plant growth regulators on barley powdery mildew caused by *Blumeria graminis* f.sp. *hordei*, under the influence of salinity, was conducted on two cultivars (Giza 123 and 126) in sand culture experiment. Plants were treated either by 10 ppm abscisic acid (ABA) or 25 and 50 ppm paclobutrazol (PBZ) as foliar spray, 3 weeks after sowing. Four levels of NaCl, *i.e.* 0, 50, 100, and 200 mM, were applied as soil treatment two days after spraying plant growth regulators. Disease severity and area under disease progress curve (AUDPC) were estimated on the leaves, at different intervals, during the experiment. Obtained data demonstrated that NaCl led to suppression of powdery mildew disease and had a harmful effect on plant growth parameters. Disease severity and AUDPC values were found to be higher in untreated control than in case of treatment by NaCl, plant growth regulators and their combinations. Treatments with PBZ and ABA significantly reduced powdery mildew severity on both cultivars and improved plant growth parameters (plant height, fresh and dry weight as well as grains yield) with increasing total chlorophyll content compared to the untreated control.

Keywords: Barley, disease severity, plant growth regulators, powdery mildew and salinity stress.

Powdery mildew is one of most destructive fungal diseases on barley (*Hordeum vulgare* L.). It caused by the biotrophic fungus *Blumeria graminis* f.sp. *hordei*, in temperate latitudes worldwide (Collins *et al*., 2002). The disease leads to a decrease in the yield and its quality (Noir *et al*., 2009). Barley is a salt-tolerant crop widely grown in the arid and semiarid regions of the Mediterranean for forage purposes and as a grain crop (Al-Karaki, 2001). Several workers used NaCl to suppress plant diseases (Eisa *et al*., 2000; Elmer, 2003 and Achuo *et al*., 2006). Eisa *et al*. (2000) indicated that infection of sugar beet by *Erysiphe betae* was reduced when the plants treated with NaCl compared to untreated.

Salinity is one of major obstacle to increase crop productivity and the most severe a biotic stress factors and today as more 800 million ha of land are salt affected on area equivalent 6% of the world (Munns, 2002), and about one-third of the world's irrigated land suffers from secondary-induced salinization (Flowers and Yeo, 1995). Tavakkoli *et al*. (2011) showed that growing barley under NaCl stress reduced the growth of barley in hydroponics and in soil with significant differences among genotypes toward the salt stress. The role of plant growth regulators (PGRs) in development of plant disease was studied by many researchers (Edwards, 1983; Michiewcz, 1987 and Al-Masri *et al.*, 2002). Alteration in the level of PGRs in disease susceptibility or resistance reaction is associated with plant pathogen interaction (Singh *et al*., 1997). Abscisic acid (ABA) plays very important role in many aspects of plant development in the regulation of stomatal aperture and in the initiation of adaptive responses to various environmental conditions. Adaptation to drought, low temperature and salinity is regulated by the combinatorial activity of interconnected ABA-dependent and ABA-independent signalling pathways (Giraudat *et al*., 1994 and Thaler and Bostock, 2004). ABA has multirole in disease resistance, where several studies reported the acquirement of plant resistance against pathogen by ABA (Ton *et al*., 2009 and Cao *et al*., 2011). Paclobutrazol (PBZ) is one of triazole compounds, a widely used as growth retardant, induces mild stress tolerance in seedlings and adult plants. More specifically, PBZ has been reported to protect the plants against drought stress (Fletcher *et al*., 2000). In addition PBS and ABA treatment increased the chlorophyll content of barley plants (El-Shamey, 2008).

The purpose of this study was to evaluate the effect of plant growth regulators as a foliar application on salinity stress and on the development of powdery mildew and growth parameters of barley plants.

Materials and Methods

Pots (30 cm diameter) were filled with washed sand (10 kg sand/pot). Five barely grains from each of tested cultivars, *i.e.* Giza 123 and Giza 126, obtained from the Wheat Res. Dept., Crop Res. Inst., Egypt, were sown in each pot (with bottom drainage) in November 2010. Pots were irrigated with tap water for the first three weeks, and then seedlings were thinned into two plants per pot. Three weeks after sowing, aqueous solutions of 25 and 50 ppm PBZ, 10 ppm ABA and tap water (Control) were foliar sprayed on plants using hand air compressed atomizer. Two days after growth regulators had been treated, the salt treatments (NaCl) were added to the water of irrigation. Pots were arranged in complete randomize design with ten replicates for each particular treatment. Each plant growth regulator treatment included four salinity levels, *i.e.* tap water (control), 50, 100, and 200 mM NaCl. Pots were amended with nutrients according to Arnon and hoagland (1940). The irrigation with the nutrient solution started two weeks after sowing and till three weeks before harvest

Inoculation and disease assessment:

The inoculation was carried out when plants reached four weeks old by shaking or brushing conidia from diseased plants on the grown plants under study. Ten days after of incubation, disease reaction was scored. This scoring was done according to a scale ranging from 0-9 according to Mastebroek *et al*. (1995). Disease severity (DS) was estimated four times during the growth period as the percentage of leaves covered by the pathogen to calculate AUDPC. The percentage of disease severity was calculated by using the following formula of Wheeler (1969):

$$\text{Disease severity (\%)} = \sum (f \times v)/nx \times 100$$

Whereas: f= Frequency of a numerical rating; v= Numerical rating scale (1-5); n= Total number of tested plants and x= maximal value (5) of the evaluation scale.

For each cultivar and each treatment, AUDPC was calculated to summarize progress of the disease according to the following formula of Shaner and Finney (1977):

$$AUDPC = \Sigma in-1[(yi+ yi+1)/2] (ti+1 - ti)$$

Whereas: n= Number of disease assessment times; y= Disease severity and t= time duration of the epidemic.

Chlorophyll content:

Plant height (cm), fresh and dry weight/plant (g) was determined 40 days after inoculation from five plants for each treatment. When the plants had reached maturity the spikes were harvested and threshed to determine the weight of the 100 grains in each treatment.

Statistical analysis:

Data were statistically analyzed by analysis of variance (ANOVA) using the statistical analysis system (SAS) software (Littell *et al.,* 1996). Means were separated by Fisher's protected least significant differences (LSD) at $P \leq 0.05$.

Results

Data presented in Table (1) clearly show that artificial inoculation of both tested barley cultivars with *Blumeria graminis* f.sp. *hordei* caused powdery mildew symptoms. Disease severity percentage of cv. Giza 123 higher than that of cv. Giza 126, being 33.3 and 29.25, respectively. The percentages of infection of plants treated with ABA (10 ppm) and PBZ (25 and 50 ppm) were 22.2, 19.25 and 16.25 % for cv. Giza 123 and 29.29, 23.33 and 15.18% for cv. Giza 126, respectively. Data show also that increasing NaCl concentration (50, 100 and 200 mM) without PGRs treatment, significantly reduced powdery mildew being 22.9, 4.40 and zero for cv. Giza 123 and 22.86, 15.18 and 11.11% for cv. Giza 126. The highest effect of NaCl and PGRs on the disease was recorded with NaCl (50 and 100 mM), where it exhibited significant reduction to the disease severity compared to untreated control. ABA (10 ppm) caused lowest effect on reducing the disease severity (Table 1). Mean PBZ (50 ppm) was the most effective one on disease severity, followed by PBZ (25 ppm). The reduction in disease severity was evident from calculated AUDPC value. AUDPC value for powdery mildew development was calculated for each tested cultivar and treatment and statistically analyzed to determine the significant differences among the treatment (Fig. 1).

Also, results indicate that there was significant difference in the AUDPC among the concentration of NaCl, ABA and PBZ for both cultivars. In addition the increase in NaCl concentration decreased plant height, fresh and dry weight and the yield of both tested cultivars (Table 2).

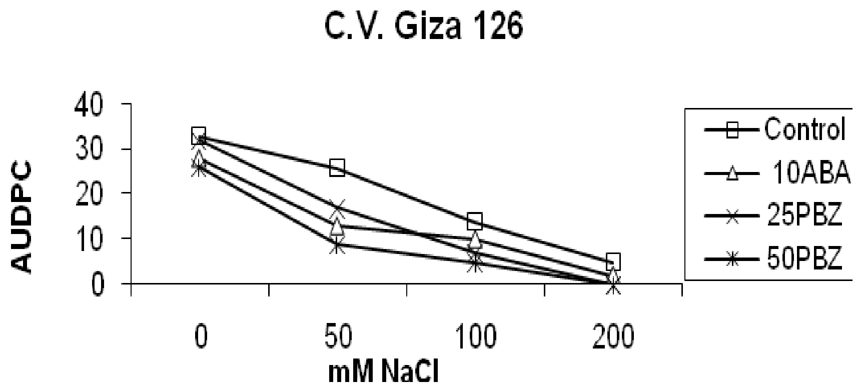
Table 1. Effect of spraying barley cvs. Giza 123 and 126 with two plant growth regulators on of the severity of powdery mildew under the influence of salinity

Treatment		Cultivar			
		Giza123		Giza126	
PGRs (ppm)	NaCl (mM)	Disease * severity (%)	Reduction (%)	Disease severity (%)	Reduction (%)
Control 0.0	0.0	33.0 **	----	29.25	----
ABA 10	0.0	22.20	23.3	29.25	0.0
PBZ 25	0.0	19.25	42.19	23.33	20.23
PBZ 50	0.0	16.29	51.17	15.18	46.05
Control 0.0	50	22.90	21.23	22.86	21.85
ABA 10	50	18.80	43.54	11.85	59.48
PBZ 25	50	15.50	52.40	11.85	60.75
PBZ 50	50	11.48	65.52	7.70	73.67
Control 0.0	100	4.40	86.78	15.18	48.10
ABA 10	100	3.70	88.88	8.51	70.90
PBZ 25	100	4.07	87.77	7.40	74.70
PBZ 50	100	0.0	0.0	3.70	87.35
Control 0.0	200	0.0	0.0	11.11	62.01
ABA 10	200	0.0	0.0	03.70	87.35
PBZ 25	200	0.0	0.0	0.0	0.0
PBZ 50	200	0.0	0.0	0.0	0.0
L.S.D. at 5 %	----	1.78	----	1.61	----

* Disease severity was recorded 40 day after inoculation.
** Average of 5 replicates.

Table 2. Effect of plant growth regulators on plant height (cm), fresh and dry weight (g) and weight of hundred grains of cvs. Giza 123 and 126, greenhouse experiment

PGRs (ppm)	NaCl mM	Plant height (cm)		Fresh weigh (g)		Dry weight (g)		Weight of 100 grain (g)	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
ABA10	0	106	118	28.4	44.5	18.7	32.0	4.20	4.9
PBZ25	0	91	98	20.0	52.6	15.6	48.0	3.32	4.6
PBZ50	0	87	97	19.4	42.2	14.0	37.1	3.14	3.6
0.0	50	92	94	11.0	17.5	7.7	15.0	2.3	2.6
ABA10	50	100	93	11.5	19.0	7.0	16.0	3.0	3.4
PBZ25	50	91	92	13.2	20.0	8.5	17.0	3.1	5.4
PB250	50	57	73	13.8	19.0	100	17.2	2.6	9.2
0.0	100	57	82	8.6	6.0	6.0	4.8	1.9	1.6
ABA10	100	84	90	12.2	9.4	9.5	8.0	1.8	2.2
PBZ25	100	89	90	12.6	19.0	11.0	17.0	2.2	2.4
PBZ50	100	75	85	12.0	16.0	8.0	15.0	1.3	1.8
0.0	200	64	61	2.6	3.0	1.6	1.8	0.4	0.4
ABA10	200	71	77	2.8	11.8	1.5	9.1	0.5	0.6
PBZ25	200	81	80	2.8	10.0	2.0	7.0	0.8	0.9
PBZ50	200	65	67	2.7	7.0	1.9	6.5	0.7	0.8
Control		105	98	35.6	36.5	29.3	28.1	3.10	3.3
L.S.D at 5 %		9.4	8.9	3.1	3.8	1.09	1.3	0.4	0.4



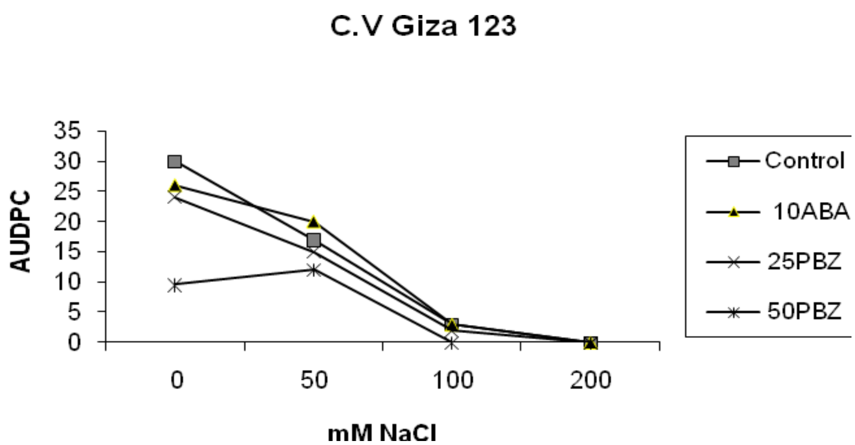


Fig. 1. Effect of different concentrations of NaCl and their combination with plant growth regulators on AUDPC of powdery mildew on cvs. Giza 123 and Giza 126.

Total chlorophyll content of the two tested cultivars showed significant decrease under salinity stress, especially at 100 and 200 mM (Table 3). PBZ treatment was more effective in increasing total chlorophyll content, being 2.62 and 2.51 mg/g fresh weight with 25 and 50 ppm when 50 mM NaCl was used on cv. Giza 126 , while it gave the least amount of chlorophyll in case of NaCl 200 mM. Untreated control recorded 0.77 and 0.73 mg/g fresh weight of cv. Giza 123 and Giza 126, respectively.

Table 3. Effect of plant growth regulators as foliar treatment, NaCl concentration and their combination on total chlorophyll content (mg/g fresh weight) of cvs. Giza 123 and 126

Conc. NaCl (mM)	Giza123				Giza 126			
	Control	ABA*	PBZ**		Control	ABA*	PBZ**	
		10	25	50		10	25	50
0	1.84	1.85	1.99	2.20	2.16	2.01	2.23	2.40
50	1.46	1.50	1.73	1.62	2.06	2.33	2.62	2.51
100	0.87	1.50	1.63	1.52	1.82	1.98	2.52	1.83
200	0.77	1.20	1.28	1.32	0.73	1.02	1.40	1.62
L.S.D at 0.05		0.21				0.29		

* Abscisic acid (ABA 10 ppm).
** Paclobutrazol (PBZ 25.5 ppm).

Discussion

The need to produce cereal cultivars tolerate salinity with disease resistance has been recognized as an important criterion in many cereal crops, especially the reduction of plant growth under salinity stress is a common phenomenon with increasing saline land in the world. The results obtained in this study clearly demonstrated that *Blumeria graminis* f.sp. *hordei*, able to infect both tested cvs. Giza 123 and 126 causing powdery mildew symptoms, but failed to do this when the plants exposed to the higher concentration of saline. The AUDPC was higher for cv. Giza 123 than cv. Giza 126 and this phenomenon may be attributed to the difference of genetic behaviour of both cultivars (Dreiseitl, 2007). The decrease in powdery mildew severity under salinity stress agreed with reported by Eisa *et al*. (2000). Some authors reported that NaCl lead to suppress of other diseases (Hopkins, 1985 and Elmer, 2003). The obtained results showed that plant growth regulators can overcome salinity problem. These results are in agreement with results reported by Achuo *et al*. (2006) and El-Shamey (2008) as they were used plant growth regulators as an antistress agent to improve salt tolerance. Results revealed, also that foliar spray by ABA and PBZ had lower AUDPC compared to untreated control. Role of plant growth regulators (PGRs) in development of plant disease resistance have been studied also by Edwards (1983), Michiewcz (1987) and Al-Masri *et al*. (2002). ABA has multirole in disease resistance, where several studies reported the acquirement of resistance against pathogens due to ABA treatment (Ton *et al*., 2009 and Cao *et al*., 2011). There are reports of positive correlation between endogenous ABA levels and plant disease resistance (Mauch-Mani and Mauch, 2005 and Achuo *et al*., 2006) via its positive effect on callus deposition or through its control of stomatal aperture and water relation. The results suggested that NaCl has damage effect on powdery mildew and on barley plants. PBZ reduced the plant height at 50 ppm, but the low concentration (25 ppm) increased the fresh and dry weight in addition to weight of the grains. PBZ (50 ppm) reduced the negative effects of increasing salinity on growth parameters and chlorophyll content. Chlorophyll content is one of many physiological processes that altered in plant grown in saline soil, which ultimately reduce their growth and yield.

Parida and Das (2005) suggested that the decrease in chlorophyll content in response to salt stress is a general phenomenon, which referred to reduction of chlorophyll synthesis and appearing of chlorosis on plants.

Results obtained in this study indicated that the ability of plant growth regulators could be used as an efficient procedure to increase plant productivity and overcome of salinity disorders as well as control of powdery mildew.

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تأثير منظمات النمو على مكافحة مرض البياض الدقيقى في الشعير تحت ظروف الإجهاد الملحي

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يُعتبر مرض البياض الدقيقى من أخطر الأمراض التى تصيب الشعير حيث يؤدى إلى نقص المحصول فى جميع مناطق زراعته. يهدف هذا البحث الى التعرف على تأثير منظمات النمو على مرض البياض الدقيقى فى الشعير وتقليل تأثير الملوحة الضار على النباتات. أجريت التجربة فى الصوبة على صنفي شعير جيزة ١٢٣ وجيزة ١٢٦ حيث رُشت النباتات بمنظمي نمو حمض الأبسيسك (١٠ جزء فى المليون) وباكلوبيوترازول (٢٥و٥٠ جزء فى المليون) وذلك بعد ٣ أسابيع من الزراعة و استخدمت أربع مستويات من الملوحة (صفر , ٥٠,١٠٠,٢٠٠ ملليمولر) وأجريت العدوى الصناعية بالمسبب المرضي ثم قدرت شدة الأصابة ومنحنى تقدم المرض (AUDPC) بعد ١٠,٢٠,٣٠,٤٠ يوم من العدوى.

أظهرت النتائج المتحصل عليها إلى إختلاف الصنفين فى القابلية للأصابة بالمرض وكانت شدة الإصابة للصنف جيزة ١٢٣ أعلى منها فى الصنف جيزة ١٢٦ كما وجد أن المعاملة بمنظمات النمو رشاً أحدثت انخفضاً معنوياً للإصابة بالمرض فى كل المعاملات المستخدم فيها منظمات النمو مع حدوث زيادة معنوية فى المحتوى الكلى للكلورفيل وأطوال النباتات و الوزن الطازج و الجاف ووزن الحبوب سوء فى وجود مستويات الملوحة أو بدونها بالمقارنة بالنباتات غير المعاملة، ووجد أن المعاملة باكلوبيوترازول أعطت حماية أفضل ضد الأصابة بالمرض مقارنة بحمض الأبسيسك. أدت المعاملة بمنظمات النمو المختلفة إلى خفض معنوي فى شدة لإصابة بالمرض وايضا زيادة فى أطوال النباتات و الوزن الطازج والجاف والمحتوى الكلي للكلورفيل ووزن الحبوب ،كما انخفض منحنى تقدم المرض (AUDPC) بارتفاع مستوى الملوحة ومنظمات النمو لكلا الصنفين وكانت أكثر الانخفاضات فى التركيزين ٥٠ و١٠٠ملليمولر.

تشير نتائج هذه الدراسة إلى امكانية استخدام بعض منظمات النمو كطريقة فعالة فى تقليل الإصابة بمرض البياض الدقيقى فى الشعير وكذلك تحسين صفاته المحصولية.